

Original Article

Effects of Bcl-I polymorphism of GR genes on the sensitivity to hormone therapy

Yu Cui^{2*}, Dongliang Shao^{4*}, Yang Zhang⁵, Deguang Liu⁶, Lei Gao¹, Hongmin Wang¹, Yingjie Hua³, Ping Jiang⁶

¹Second Department of Pediatrics, Departments of ²Pediatrics, ³Emergency Pediatrics, People's Hospital of Linzi District, Zibo, Shandong Province, China; ⁴Department of Pediatrics, People's Hospital of Jinxiang, Jining, Shandong Province, China; ⁵Department of Pediatrics, Laoling People's Hospital, Dezhou, Shandong Province, China; ⁶Department of Pediatrics, Shandong Sunshine Union Hospital, Weifang, Shandong Province, China.
*Equal contributors and co-first authors.

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Abstract: Objective: To study the correlation of Bcl-I locus polymorphism of glucocorticoid receptor (GR) genes with the onset of pediatric asthma and glucocorticoid (GC) sensitivity in children. Methods: A total of 336 asthmatic children were enrolled as an observation group, and treated with GC, and 300 healthy children in physical examination during the same period were enrolled as a control group. They were analyzed prospectively. Mouth mucosa exfoliative cells were sampled from them, and the DNA of the cells was extracted. The Taqman probe method was employed for single nucleotide polymorphism (SNP) locus typing to the Bcl-I locus polymorphism of GR genes, and the plasma cortisol (Cor) level was also determined. The genotype frequencies and allelic frequency of Bcl-I in GR genes were compared between the two groups, and the treatment efficacy, and plasma Cor level, and hormonal resistance incidence were compared between children with different genotypes. Results: The observation group showed higher GG and GC genotype proportions at the Bcl-I locus of GR genes than the control group (both $P < 0.05$), and also showed a higher G allele base frequency than the control group ($P < 0.01$). In the observation group, the plasma Cor levels of the three genotypes were not significantly different ($P > 0.05$), and the total treatment efficacy rate in the asthmatic children with GG or GC genotype was lower than that in those with CC genotype ($P < 0.05$), and the hormonal resistance incidence in the asthmatic children with GG or GC genotype was higher than those with CC genotype ($P < 0.05$). Conclusion: Single nucleotide polymorphism caused by mutation of base C to G at Bcl-I locus of GR genes in asthmatic children is closely related to the onset of asthma, and it can enhance the risk of glucocorticoid resistance and compromise its efficacy. The G allele is a susceptible gene for disease onset and hormone resistance, but no obvious correlation between plasma Cor level and hormone resistance is found.

Keywords: Children, asthma, GR gene, Bcl-I polymorphism, hormone therapy, sensibility

Introduction

Asthma is a common respiratory disease in childhood, and a chronic inflammatory disease of the airway mediated by cells such as mast cells, neutrophils, and T cells, and their components. It is accompanied by persistent and reversible airway hyper-responsiveness and remodeling, and is mainly clinically manifested as repeated cough, allergic asthma, and dyspnea [1]. The etiology of pediatric asthma is complex, which involves many factors such as heredity, environment, allergy, infection, and immunity, and is characterized by multi-cause and heterogeneity. Among those factors, hered-

ity is particularly closely related to the onset of pediatric asthma according to studies. Epidemiological statistics show that the incidence of asthma among children aged 0-14 years in China has increased from 1.08% in 1990 to 3.01% in 2010 during the past 20 years, which increased by nearly three times [2]. Therefore, the early diagnosis and treatment of asthma has become a major concern in pediatrics.

At present, pediatric asthma is mostly treated as follows in clinical practice: rapid symptom relief and anti-inflammation and antiasthma for pediatric asthma in the acute phase, and long-

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term anti-inflammation, alleviation of airway inflammation and avoidance of inducing factors in the persistent phase. Glucocorticoid (GC) is the first choice and an important therapeutic drug for treatment, which exerts anti-inflammatory, antitussive, antiasthmatic effects and other effects through the glucocorticoid receptor (GR) [3]. However, a study has found that some children still suffer from unsatisfactory symptoms and airway hyper-responsiveness after being treated with GC shock therapy for long term or with a large dose, and they may even show disease relapse and deterioration in the case of sudden withdrawal of hormone, and develop GC resistant asthma. Based on the important position of GC in pediatric asthma [4], and the significant increase in the incidence of hormone-resistant asthma, the research on GC resistance in pediatric asthma has made important progress. A study has found that the efficacy of GC in vivo is not only related with dosage, but also greatly affected by it [5]. The most important point is that GR activity is directly affected by GR gene polymorphism. However, there are few reports on the correlation of GR gene polymorphism with the onset of pediatric asthma and GC resistance. Therefore, this study determined the GR gene polymorphism in healthy children and asthmatic children, and analyzed the correlation between GC resistance and GR gene polymorphism in asthmatic children, so as to provide theoretical guidance for genetic susceptibility and treatment of pediatric asthma.

Materials and methods

General data

A total of 336 asthmatic children admitted to People's Hospital of Linzi District were selected in this study, and enrolled as an observation group. The inclusion criteria were as follows: children meeting relevant diagnostic criteria of the 2016 *Guidelines for diagnosis and management of childhood bronchial asthma* [6], children received hormone therapy and meeting therapeutic indications, children without comorbid severe diseases in organ tissues such as heart, brain, blood vessel, liver, or kidney, children between 2 and 12 years old, children without family heredity history, and being treated for the first time. The exclusion criteria were as follows: patients with a comorbid respi-

ratory disease such as chronic obstructive pulmonary disease, pneumonia, and pulmonary tuberculosis, patients who had received GC treatment for a long term, patients with a comorbid disease such as lupus erythematosus, psoriasis and nephrotic syndrome, patients with a comorbid allergic disease such as atopic dermatitis and anaphylactoid purpura. A total of 300 healthy children in physical examination during the same period were enrolled as a control group. All children's families voluntarily participated in the study, and signed informed consent forms, and the study was examined and approved by the Ethics Committee of the hospital.

Methods

Treatment methods

Children in the observation group were treated with GC and methylprednisolone (Shanghai General Pharmaceutical Co., Ltd., H31021289, 5 mL/0.125 g). Methylprednisolone was intravenously injected at 1-2 mg/(kg.d) for 7 consecutive days, with gradual dosage decrease to drug withdrawal. 1.2.2 Analysis on Bcl-I locus polymorphism of GR genes Whole blood genomic DNA was sampled from children in the observation group and in the control group in accordance with the *Molecular cloning: a laboratory manual*, and all samples were placed at -20 C for later analysis. The Taqman probe method was employed for gene locus sequencing. The Taqman probe was designed and synthesized by the Applied Biosystems Company in the United States based on RS serial number of Bcl-I locus of GC genes. The probe and primers used in the Taqman probe method for sequencing were also designed and synthesized by the company, which included two primers and a Taqman MGB probe. The later was labeled with VIC and FAM, respectively. Then PCR amplification was carried out with an ABI9700 PCR amplification instrument under a reaction system consisting of 10.0 µL of double distilled water, 2.0 µL of DNA, 2.0 µL of dNTP, 2.5 µL of 10× Buffer, 20.0 µL of MgCl, 0.25 µL of Tap enzyme, 1.0 µL of upstream primers, and 1.0 µL of downstream primers, and 0.2 µL of probe. The primers were designed using Primer software on line. The upstream primer sequence was 5'-GCGAAATTCAC-CCGTACCAAC-3', and the downstream primer sequence

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Table 1. Comparison of baseline data between control group and observation group

Group	Sex (Male/Female)	Mean age (year)	BMI
Control group (n=300)	189/111	2~12 (5.26±2.61)	16.75±2.1
Observation group (n=336)	192/144	3~12 (5.33±2.57)	16.98±1.5
t/χ ²	1.842	0.977	1.602
P	>0.05	>0.05	0.110

Note: BMI, body mass index.

was 5'-GGCAGGGTTG-AGCGCCGATG-3'. The primers were synthesized by Shanghai Generay Biotech Co., Ltd. The reaction conditions: pre-denaturation at 94 C for 5 min, followed by 35 cycles of denaturation at 94C for 45 s, annealing at 58C for 1.5 min, and extension at 72 C for 5 min, and then another extension at 75 C for 10 min. Restriction enzyme cutting of PCR products: 10.0 μL of the product was selected, and added with 1.2 μL of Bcl-I restriction enzyme, 2.0 μL of 10× Load-ing Buffer, and 6.0 μL of distilled water, followed by a water bath at 37C for 10 h. Electrophoresis: 1.0% agarose gel was used for electrophoresis under a voltage controlled at 120 V for 20 min, and PCR Markers were set as reading reference. Imaging was achieved using a ZFAI ultra-violet transmission analyser (Shanghai Guangming Optical Electronic Instrument Factory), and the final genotype results were recorded by the ABI SDS2.3 software.

Determination of plasma cortisol (Cor) level

Fasting peripheral venous blood (3.0 mL) was sampled from children in the observation group, and the plasma Cor level of the samples was determined using the radioimmuno assay with a UniCelDxl 800 immunoassay system (Beckman Coulter Trading (China) Co., Ltd.). The kit was provided by Shanghai Heng Yuan Biotechnology Co., Ltd, and all steps were strictly carried out with reference to the operating instructions.

Observation indexes

The following aspects of the two groups were compared: the GG, GC, and CC genotypes, and allele frequencies of G and C at Bcl-I locus of GR genes, and plasma Cor levels of GG, GC and CC genotypes. The total treatment efficacy rate and hormone resistance incidence were compared in children with GG, GC, or CC genotype

in the observation group. The diagnostic criteria for hormone-resistant asthma: after oral administration of prednisolone (≥20 mg/d) for more than one week, the improvement rate of forced expiratory volume in 1 second (FEV₁) is still ≤15.0%.

Statistical analysis

In this study, all statistical data were processed using SPSS21.0. All measurement data were expressed by ($\bar{x} \pm sd$). Comparison between the two groups was carried out using the t test, and post hoc comparison among three groups and above was conducted using the one way ANOVA and LSD successively. Enumeration data were expressed by the number of cases/percentage (n/%). Inter-group comparison was performed using χ^2 . Unconditional logistics regression was adopted to analyze genotypes and the risk of asthma. P<0.05 indicates a significant difference.

Results

Comparison of baseline data between the two groups

There were no significant differences between the two groups in the general data of sex and age (both P>0.05). Details are shown in **Table 1**.

Electrophoresis of PCR amplification products at Bcl-I locus of GR genes

Normally, Bcl-I locus of GR gene has a restriction enzyme cutting locus, but when weak allele C mutates into G, it has no restriction enzyme cutting site. Namely, there are two electrophoresis bands, 151 bp and 267 bp, after wild-type CC cleavage, and there are three electrophoresis bands, 151 bp, 267 bp, and 418 bp, after mutant heterozygote GC genotype cleavage, while there is only one electrophoresis band, 418 bp, after mutant homozygote GG genotype cleavage. Details are shown in **Figure 1**.

Analysis on Bcl-I locus polymorphism of GR genes of the two groups

The GG genotype frequency of the children from the observation group was significantly

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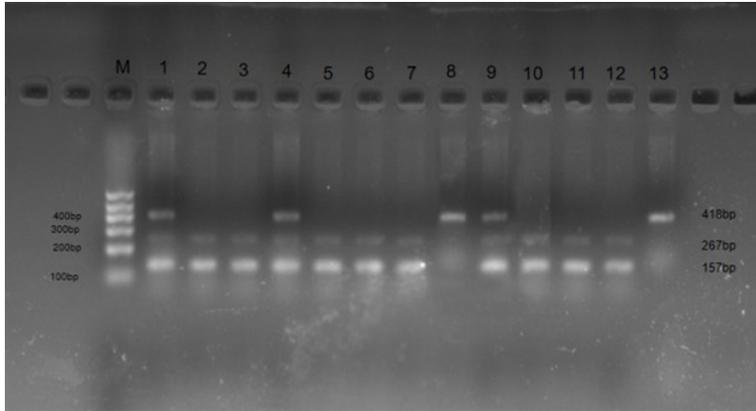


Figure 1. Electrophoresis of PCR amplification products at Bcl-I locus of GR genes. Note: GR, glucocorticoid receptor.

higher than that of the children from the control group (30.95% vs. 3.00%, $P < 0.01$), and the G gene frequency of the children from the observation group was also significantly higher than that of the children from the control group (42.11% vs. 13.00%, $P < 0.01$). Details are shown in **Table 2**.

Comparison of plasma Cor levels between the two groups with different genotypes

In both groups, there was no significant difference in the plasma Cor level among the CC group, GC group and GG group (all $P > 0.05$), but the plasma Cor level of the GG group was significantly higher than that of the GC group and CC group (both $P < 0.01$). Details are shown in **Table 3**.

Comparison of effective treatment rate between children with different genotypes in the observation group

In the observation group, the total treatment efficacy rate of CC group was significantly higher than that of the GC group and GG group (both $P < 0.05$), and that of the GC group was significantly higher than that of the GG group ($P < 0.05$). Details are shown in **Table 4** and **Figure 2**.

Comparison of the hormonal resistance incidence between children with different genotypes in the observation group

The hormonal resistance incidence in the children with GG genotype is significantly higher than that in the children with GC genotype and

the children with CC genotype (66.35% vs. 48.00% vs. 18.47%, respectively; all $P < 0.05$). Details are shown in **Table 5**.

Discussion

GC is the first choice and most effective drug for asthma at present, and GR is the basis for the anti-inflammatory function of GC [7-10]. However, some patients still show unsatisfactory disease control after inhaling GC even after long-term inhalation at a

large dosage, and suffer from hormone-resistant asthma. The specific mechanism of hormone-resistant asthma is very complex, which is related to many factors such as heredity, key gene mutation, GR number and abnormal affinity [11, 12]. With a further genome-wide association study (GWAS), multiple polymorphic local of asthma susceptibility genes have been found one after another, and the correlation of GR genes with the onset of pediatric asthma and hormone resistance has drawn a high attention.

The results of this study showed that the total treatment efficacy rate for GG genotype at Bcl-I locus of GR genes was significantly lower than that for GC genotype and CC genotypes in the observation group, and the hormonal resistance incidence in children with GG genotype was 66.35%, dramatically higher than that of those with GC or CC genotype. The Bcl-I locus polymorphism of GR genes was first found in studies on obesity, and it was found that mutation from allele C to G would increase the insulin resistance level of patients. Then a study on this was successively carried out in diseases such as airway obstruction, pulmonary fibrosis, and airway variation, and it was found that the mutation from allele C to G of Bcl-I locus could accelerate the progression of those diseases [13-15]. A study by Rogausch et al. pointed out that smoking in patients with Bcl-I GG genotype suffered significantly greater airway obstruction than those with CC genotype, and having the G allele was a high-risk gene affecting airway variation [16]. A study by Corvol et al. found that the Bcl-I locus polymorphism could affect

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Table 2. Analysis on Bcl-I locus polymorphism of GR genes of the two groups [n (%)]

Bcl-I locus of GR genes	Control group (n=336)	Observation group (n=336)	OR	95.0% CI	P
Genotype					
CC	157 (46.73)	231 (77.00)	0.943	0.748~1.473	0.000
GC	75 (22.32)	60 (20.00)	1.436	0.352~0.637	0.646
GG	104 (30.95)	9 (3.00)	0.874	0.581~0.839	0.002
GG+GC	179 (53.27)	69 (23.00)	0.905	0.436~2.469	0.000
Allele					
G	283 (42.11)	78 (13.00)	0.849	0.702~1.741	0.003
C	389 (57.89)	522 (87.00)	0.792	0.476~1.843	0.007

Note: GR, glucocorticoid receptor; CI, confidence interval; OR, odds ratio.

Table 3. Comparison of plasma Cor levels between the two groups with different genotypes ($\bar{x} \pm sd$)

Group	plasma Cor levels (ng/mL)			F	P
	CC group (n=231, 157)	GC group (n=60, 75)	GG group (n=9, 104)		
Control group (n=300)	105.47±50.22	122.86±52.07	143.79±42.80	5.394	0.000
Observation group (n=336)	114.96±43.87	126.92±49.61	135.43±44.62	6.659	0.000
t	2.492	1.058	1.880	-	-
P	0.127	0.632	0.373	-	-

Note: Cor, cortisol.

Table 4. Comparison of effective treatment rate between children with different genotypes in the observation group [n (%)]

Group	Clinical control	Excellence	Effective	No of effect	Total effective rate (%)
CCgroup (n=157)	46 (29.30)	70 (44.58)	21 (13.38)	20 (12.74)	87.26
GC group (n=75)	12 (16.00) ^{&}	19 (25.33) ^{&}	15 (20.00)	29 (38.67) ^{&}	61.33 ^{&}
GG group (n=104)	10 (9.62) ^{&#}	16 (15.38) ^{&#}	17 (16.35)	61 (58.65) ^{&#}	41.35 ^{&#}
Z			-		9.572
P			-		0.000

Note: [&]compared with CC group, P<0.05; [#]compared with GC group, P<0.05.

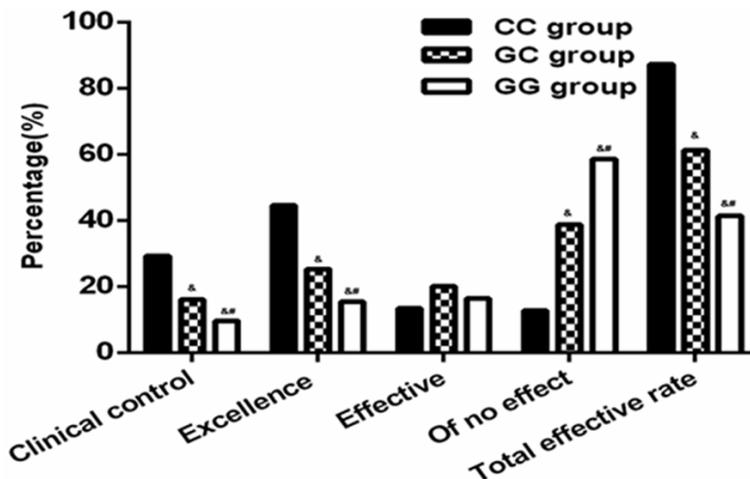


Figure 2. Comparison of effective treatment rate between children with different genotypes in the observation group. Compared with CC group, [&]P<0.05; Compared with GC group, [#]P<0.05.

the severity of lung function damage in patients with pulmonary fibrosis, and the patients with GG genotype showed more significantly lowered forced expiratory volume (FEV) and forced expiratory volume in 1 second (FEV1) than those with GC genotype and CC genotype, and were more reactive to GC [17]. Therefore, it was speculated that allele G of Bcl-I locus of GR genes may affect the progression of inflammatory reaction, thus reducing the sensitivity of patients to GC. Although there are few reports

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Table 5. Comparison of the hormonal resistance incidence between children with different genotypes in the observation group [n (%)]

Group	Hormonal resistance	Hormonal sensitivity	No hormonal resistance or sensitivity
CC (n=157)	29 (18.47)	102 (64.97)	26 (16.56)
GC (n=75)	36 (48.00) ^{&}	27 (36.00) ^{&}	12 (16.00)
GG (n=104)	69 (66.35) ^{&,#}	16 (15.38) ^{&,#}	19 (18.27)
Z	6.426	7.615	1.041
P	0.000	0.000	0.527

Note: [&]compared with CC group, P<0.05; [#]compared with GC group, P<0.05.

on the sensitivity of genotypes to hormone therapy, the above results all suggest that mutation from allele C to G has adverse effects on respiratory diseases or hormone sensitivity. This study has confirmed that the mutation from allele C to G at Bcl-I locus would increase the risk of hormone resistance, but its specific molecular mechanism remains unclear. We believe that allele mutation can cause abnormal expression of GR receptor protein structure and function, and down-regulate the GR expression number and hormone affinity by lowering the gene transcription activity and changing protein coding types, thus resulting in hormone resistance [18-20]. There was no significant difference in plasma Cor levels of different genotypes in the two groups. It suggested that the secretion and metabolism of endogenous cortisol hormone were normal, and the effect of adrenocortical function difference on GC sensitivity can be ruled out, which provided a more sufficient basis.

The results of this study revealed that the GG genotype frequency of the children in the observation group was dramatically higher than that of the children in the control group (30.95% vs. 3.00%), and the G gene frequency of the children in the observation group was also dramatically higher than that in the control group (42.11% vs. 13.00%). It suggested that the allele G of Bcl-I locus may be the susceptible gene of pediatric asthma, and allele mutation may cause abnormal expression of related proteins, thus increasing the risk of asthma. Bcl-I locus is located at the 646 nucleotide downstream of GR gene exon 2, and wild-type C base mutation due to G base (TGATCA to TGATGA) forms two fragments with different lengths, 3.9 kb and 2.2 kb, which is the main reason for

single nucleotide polymorphism of this locus. There have been preliminary studies abroad on the correlation of Bcl-I locus polymorphism of GR genes and asthma, but there is still a lack of such studies in China. A study by Pietras et al. found that the GG genotype frequency at Bcl-I locus of GR genes in asthma patient was significantly higher than that in the healthy people from the control group (41.0% vs. 12.9%), and mutation of allele C to G would affect the transcription activity of GR genes,

down-regulate GR expression number and hormone affinity, increase hormone resistance risk, and enhance asthma susceptibility [21]. However, another study has also found that there is no obvious correlation of Bcl-I locus polymorphism with asthma susceptibility and GC reactivity [22].

However, the onset of asthma is related to many factors such as genes and environment [23], and there are many kinds of related genes, so there are limitations in studying the polymorphism of one gene. At the same time, GR gene belongs to a family of drug target genes, and the results of gene polymorphism may be affected by drug therapy. Therefore, we need to further control the interference of non-experimental factors, increase the sample size and the number of research genes to further and accurately study the correlation of asthma onset and treatment with gene polymorphism.

To sum up, single nucleotide polymorphism caused by mutation of base C to G at Bcl-I locus of GR genes in asthmatic children is closely related to the onset of asthma, and it can enhance the risk of glucocorticoid resistance and compromise its efficacy. G allele is a susceptible gene for disease onset and hormone resistance, but no obvious correlation between plasma Cor level and hormone resistance is found.

Disclosure of conflict of interest

None.

Address correspondence to: Ping Jiang, Department of Pediatrics, Shandong Sunshine Union Hospital, No. 9000 Yingqian Street, High-Tech Dis-

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tract, Weifang 261000, Shandong Province, China.
Tel: +86-0536-6960982; Fax: +86-0536-6960982;
E-mail: jiangping2jp8@163.com

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